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Morphological characterization and epidemiological studies of summer squash (*Cucurbita pepo*) powdery mildew

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ABSTRACT

Samples of powdery mildew of summer squash (*Cucurbita pepo* L.) collected from Solan, Shimla, Hamirpur and Kinnaur districts of Himachal Pradesh during 2017–18 revealed the presence of anamorphs, whereas the cool and dry temperate region of district Kinnaur showed the occurrence of both teleomorphic and anamorphic stages of powdery mildew on summer squash. Present studies constitute the first report of cleistothecial occurrence on summer squash under Indian conditions. Based on anamorphic characters like ellipsoidal conidia, presence of fibrosin bodies and germinating conidia produced both simple and forked germ tubes without appressorium and teleomorphic characters like cleistothecia with myceloid appendages, single ascus with eight ascospores the associated pathogen was identified as *Sphaerotheca fuliginea* (Schlecht.) Pollaci. Maximum conidial germination was observed at 25° temperature and 100% relative humidity. The development of powdery mildew disease was significantly favored at moderate temperature coupled with high relative humidity and cumulative rainfall.

Keywords: Anamorphs, Epidemiology, *Cucurbita pepo, Sphaerotheca fuliginea,* Teleomorphs

Summer squash (*Cucurbita pepo* L.) is one of the important cucurbitaceous vegetable crops grown throughout world. It was originated in Central America, Mexico and Northern parts of South America (Formisano *et al.* 2012). In India, the area under pumkin, squash and guards is 574 thousand ha and production is 5506 thousand tonnes (FAO 2018). However, the area under summer squash is about 5.68 thousand ha with 11.46 thousand tonnes production (FAO 2017). During its cultivation, the crop is severely affected by powdery mildew disease. World over, the disease has been known to be caused by three pathogens, viz. *Sphaerotheca fuliginea, Erysiphe cichoracearum* and *Leveillula taurica* (He *et al.* 2017) but from Himachal Pradesh only *E. cichoracearum* has been reported to be the causal agent of this disease (Gupta *et al.* 2017). Occurrence of teleomorphic stage of few powdery mildew genera had been reported in dry temperate zone of Himachal Pradesh (Bharat 2009). The teleomorphic i.e., cleistothecial stage is not of very common occurrence and hence anamorphic characters are usually used for identification of the powdery mildew pathogen (Félix-Gastélum *et al.* 2005). The teleomorphic stage of powdery mildew of summer squash is not yet reported from India. The present studies were therefore, carried out on occurrence and characterization of anamorphic and teleomorphic stage of the pathogen. Further, role of meteorological factors in the development of the disease both under laboratory and natural conditions was also studied.

MATERIALS AND METHODS

Collection of samples and identification of pathogen: The samples of diseased plant parts were collected from different areas such as Solan (Nauni, Oachghat, Kandaghat, Shamti, Sproon), Shimla (Taradevi, Shoghi, Khalini), Hamirpur (Neri, Bhota, Rangus) and dry temperate region of Kinnaur district (Sharbo, Kalpa) of Himachal Pradesh during 2017–18. Samples were brought to the laboratory and examined under microscope to detect the presence of anamorphs and teleomorphs of the pathogen. Teleomorphs were only recorded in the samples collected from Kinnaur area of the state. Morphological characters of anamophic stage of the powdery mildew fungus were studied microscopically according to Diego and Do (2010). Teleomorphic characters like presence of cleistothecia, type of appendages, number of asci/cleistothecium and number of ascospores/ascus were also studied.

Effect of temperature and relative humidity on conidial

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germination: Conidia from freshly sporulating mildew colonies were gently dusted on clean glass slides with the help of small, motorized air pump used for aerating aquariums to get air pressure for blowing off conidia (Thind *et al.* 1996). For temperature studies, the slides were kept in Petri-plates (90 mm dia.) containing moistened cotton wool and were transferred to incubators maintained at 15, 20, 25, 30 and 35°C while for relative humidity studies, slides were kept in humidity chambers maintaining different levels of relative humidity (Gupta *et al.* 2001). All the treatments were replicated four times. The petri-plates were sealed with parafilm and subsequently transferred to an incubator maintained at 25±1°C. After 24 h of incubation, slides were microscopically examined to record conidial germination and germ-tube length.

Effect of meteorological factors on disease development under field condition: A field experiment was laid out at the experimental farm of the department at Nauni, Solan during 2017 and 2018. Plants of summer squash were raised following the standard package and practices but without any fungicidal application. The data on disease severity of powdery mildew was recorded according to 0-5 scale described by Kamel *et al.* (2018) at three days interval commencing from 24 May and continued up to 3 July during two consecutive crop seasons, i.e. 2017 and 2018. Meteorological data on relative humidity, cumulative rainfall, sunshine hours and temperature for the prevailing period between disease data recordings were collected from Department of Environmental Science of the University. Simple, partial and multiple correlations between disease severity and four independent meteorological factors were calculated.

RESULTS AND DISCUSSION

Identification of the pathogen: All the samples collected from different locations contain anamorphic stage of the powdery mildew pathogen. However, samples collected from Kinnaur yielded both anamorphic and teleomorphic stages. The analysis of observations on anamorphic characters revealed the presence of ectophytic mycelium, aseptate and unbranched conidiophores with a cylindrical foot cell. Conidia had ellipsoidal shape and were arranged in long chains with basipetal succession. The average conidial size was about 31.53 × 20.34 μm. The value of Length /Breadth (L/B) index was less than 2 (mostly 1.5). The fibrosin bodies were present in about 89.84% conidia and average number of fibrosin bodies per conidium was 5.42. The conidial germination was 49.92%. Studies on conidial germination revealed that the conidia either germinate by forming simple germ-tube or forked germ-tube. The 42.78% conidia germinated with simple germ tube while 57.22% germinated with forked germ-tube. The germ-tube emerged laterally from the sidewall of the conidium. Formation of appressoria was not observed in all the examined samples. These findings are in agreement with the findings of earlier workers and were successfully used for the identification of the causal agent of powdery mildews (Bharat 2009, Gupta *et al.* 2017). However slight variations in conidial dimensions were exhibited in the present study. Differences in conidial dimensions as observed in the present studies are of significance and suggest the existence of a different race of the pathogen in the area.

Formation of cleistothecia was observed in Kinnaur area of state. The cleistothecia were either scattered or densely gregarious. They are globular in shape and brown in colour. The average size of cleistothecium was 79.59 × 73.42 μm. The appendages on cleistothecia were as long as the diameter of the cleistothecium and number of appendages were variable. Further, appendages were myceloid, brown in color and were attached at the base of the cleistothecium. A single ascus per cleistothecium was observed. Asci were elliptical and subglobose with average dimension of 51.94 × 40.71 μm. Each ascus contained eight ascospores which were ellipsoid to nearly spherical in shape with average size of 12.76 × 10.36 μm. Based on the above mentioned anamorphic and teleomorphic characters, the associated pathogen was identified as *Sphaerotheca fulginea* (Schlect.) Pollaci which is in consonance with earlier workers (Perez-Garcia *et al.* 2009, Pirondi *et al.* 2015).

The cleistothecial production is rare in nature and there is no report of cleistothecial occurrence on summer squash under Indian conditions. However, the occurrence of cleistothecia on other cucurbits has been reported (Gupta and Sharma 2012, Agnihotri *et al.* 2014). This is the first report of occurrence of cleistothecia on summer squash from dry temperate zone of Himachal Pradesh. Studies on effect of different temperature levels on conidial germination revealed that conidial germination was not favoured at very high or low temperature. Maximum conidial germination of 44.06% was observed at 25°C which was followed by 37.92, 33.96 % at 30°C and 20°C, respectively. These results are in accordance with Trecate *et al.* (2019) and Sapak (2012). The length of germ-tubes varied from 8.71–19.78 μm at different temperature regimes with maximum at 25°C. The optimum temperature range of 20–26°C for conidial germination and germ-tube growth for most of powdery mildew pathogens have been reported (Gupta *et al.* 2017).

Effect of different humidity levels was evaluated for conidial germination. Maximum conidial germination of 52.62% was observed at 100% relative humidity. It was followed by 46.94 and 43.77 % conidial germination at 99.1 and 98.2 % relative humidity, respectively. The conidia did not germinate below 89.9% relative humidity. Further, observations on germ-tube length showed that there was huge difference in germ-tube length which ranged between 0.10 to 27.91 μm. These findings are in accordance with Rana *et al.* (2018) who reported that high relative humidity was required for the germination of conidia of *S. fulginea*. The analysis of meteorological factors and infection of powdery mildew on summer squash showed that the disease initiates in second fortnight of May and attained maximum level in July (Fig 1). Further, it was also found that relative humidity was essential for the initiation of the disease. The statistical analysis on simple correlation showed that there is

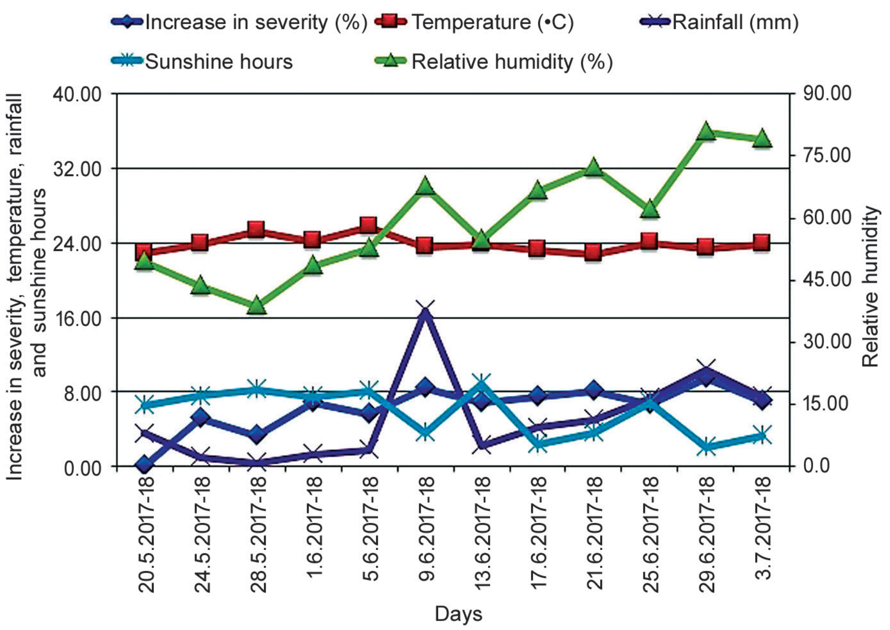

Fig 1 Influence of meteorological factors on the development of powdery mildew of summer squash during 2017 and 2018 crop seasons at Nauni, Solan.

Table 1 Pooled simple and partial correlation coefficients between disease severity and meteorological factors during 2017-18 at Nauni, Solan

Meteorological factor	Increase in disease severity (%)	
	Simple	Partial
Temperature	-0.747**	-0.551*
Relative humidity	0.851**	0.548*
Rainfall	0.723**	0.415
Sunshine hours	-0.761**	0.217

*Significant at 10 %; **Significant at 5 %

a positive and highly significant correlation between disease severity and mean relative humidity and between disease severity and cumulative rainfall (Table 1). Prevalence of severe powdery mildew disease under high humidity had also been reported earlier (Pathania and Gupta 2016, Rana *et al.* 2018). However, the correlation of disease severity with mean temperature (-0.747) and sunshine hours (-0.761) were found to be negative and highly significant. Similar findings had been reported in cucumber powdery mildew (Gupta *et al.* 2001). However, Pugliese *et al.* (2012) had reported that powdery mildew of cucumber was favored by high temperature. In contrast during the present studies, significant but a low negative correlation was observed between disease severity of powdery mildew of summer squash and mean temperature.

Partial correlation between disease severity and relative humidity was found to be positive with a value of 0.548 but non-significant during 2017 and 2018. Similarly, rainfall showed (0.415) positive and non- significant partial correlation with disease severity for both years, while that of temperature with disease severity were found to be negative (-0.551) and non-significant. Analogically, there was a positive (0.217) but non-significant partial correlation between total sunshine hours and severity of powdery mildew disease for two consecutive years. The multiple correlation coefficient between disease severity and group of independent variables was found to be 0.829, which indicated that 82.9% change in disease severity was caused by mean temperature, cumulative rainfall, mean relative humidity and sunshine hours collectively, whereas the rest of the variation was due to unexplained factors (error variation) and the factors not included in the investigations. The multiple regression equation $Y_1 = 20.145-0.784X_1+0.069X_2+0.090X_3+0.130X_4$, where Y_1 = increase in disease severity (%), X_1 =Mean temperature, X_2 = Mean relative humidity, X_3 = Cumulative rainfall, X_4= Sunshine hours, showed that unit change in relative humidity could influence the disease severity up to an extent of 0.069, followed by cumulative rainfall (0.090) and sunshine hours (0.130) in the positive direction, while mean air temperature (-0.784) affected negatively. Moreover, partial correlations and regression equation between disease severity and environmental factors have further elaborated the role of mean relative humidity and sunshine hours for disease development. These results corroborate the findings of Gupta *et al.* (2001) who reported higher incidence of powdery mildews to higher humidity besides, low temperatures and reduced light intensity.

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